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Effect of miswak varnish on enamel demineralization in orthodontic patients (a randomized controlled study)

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Abstract

Background: Enamel demineralization and white spot lesion development are prevalent side effects in patients undergoing fixed orthodontic appliance treatment. **Aim:** To investigate the effect of miswak varnish on the incidence and severity of enamel demineralization around orthodontic brackets compared to fluoride varnish, and to investigate the combined effect of both miswak and fluoride varnishes on enamel demineralization around orthodontic brackets.

Material and methods: Brackets were bonded in eligible participants who were equally allocated in one of 3 groups at random.

• (FV group): Fluoride varnish (Fluoro-protector, Ivoclar-Vivadent, Schaan, Liechtenstein) was applied to teeth surface around orthodontic brackets.

• (MV group): Freeze dried aqueous miswak extract varnish was applied to teeth surface around orthodontic brackets.

• (CG group): Both fluoride and miswak varnishes were applied to teeth surface around orthodontic brackets.

After one month, maxillary first premolars were extracted in every patient and sectioned buccolingually. Enamel microhardness was evaluated in each half crown in 8 different positions. One point under the bracket (as a control), one point at each edge of the bracket (0µm) occlusal and cervical (total 2 points), one point at 100µm distance from the bracket edge in both occlusal and cervical direction (total 2 points), one point at 200µm distance from the bracket edge in both occlusal and cervical direction (total 2 points) and one point was assigned in the middle third of the lingual surface of each half crown (as control). The mean of microhardness was tested at each assigned point at the distances of 10, 20, 30, 50,70 and 90 µm from the enamel surface.

Results: Statistically significant difference between test groups in enamel demineralization was observed at 10µm, 20µm and 30µm from enamel surface. The combined group showed the least demineralization followed by the fluoride group. The miswak group had the greatest amount of enamel demineralization.

Conclusion: Miswak varnish has a synergistic effect to fluoride varnish in enamel protection against demineralization.

Introduction

Enamel demineralization and white spot lesion (WSL) development are prevalent

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side effects seen in almost half the patients undergoing fixed orthodontic appliance treatment (1,2).

Fixed orthodontic appliances interfere with proper oral hygiene measures, resulting in greater plaque accumulation on the bonded tooth surfaces. Dietary carbohydrates fermentation by plaque bacteria produce acids that demineralize enamel leading to mineral loss from enamel surface and is manifested clinically as white spots (3,4) Streptococcus mutans *(S.mutans)* is the main cariogenic bacteria involved in the development of dental caries. *S.mutans* is primarily responsible for plaque formation on tooth surface due to their ability to build and aggregate the extracellular glucans(5,6). *S. mutans* is responsible also for dietary carbohydrates metabolism to produce lactic acid. Moreover, The bonding of fixed orthodontic appliance is responsible for an increase in salivary and plaque *S.mutans* counts (7–10).

Prevention of carbohydrate fermentation and acid production either by reducing plaque bacterial counts or preventing its metabolic activity has been proved an efficient caries control strategy(11). This strategy could be achieved by different methods, however finding a strategy that is independent on patient compliance is preferable, as only 13% of orthodontic patients could obey a preventive program for continuous 1 or 2 years(2).

Dental varnishes are good example for a patient-independent method used to achieve an anti-caries strategy. Varnishes could be applied every 3 to 6 months by the dentist or by his assistant(12,13). Varnishes contain high concentration of the constituent active ingredient and transfer it to the surface they are applied to(14). The most used varnish in dental practice is fluoride varnish. Fluoride varnishes have proved efficiency in decreasing enamel demineralization with 40% to 50% lesion depth reduction(2,15,16).

Fluoride can promote remineralization by forming CaF_2 crystals that release fluoride during low PH levels and are more resistant to acid attack(11,17). Fluorides also can minimize demineralization by decreasing lactic acid production by cariogenic bacteria(18). This happens by interfering with bacteria metabolism rather than being fluoride an antimicrobial agent(14).

The use of antimicrobial agents along with fluorides have proved a synergistic effect and improved the cariostatic effect of fluoride(19,20). Some guidelines recommended the combined use of fluoride and antimicrobial agent such as xylitol or chlorhexidine in high risk individuals to reduce the incidence and severity of dental caries (21,22). Among the different known antimicrobials, chlorhexidine has been considered the gold standard antimicrobial used in oral health(23–27). Chlorhexidine reduce bacterial counts in dental plaque(23) and consequently reduces the pH fall during demineralization episodes(28). An in vitro study investigated the effect of fluoride varnish on *S. mutans* and *S. sobrinus* counts and found that the highest bacterial counts was found when the fluoride varnish was used alone, however, a combination of fluoride and

chlorhexidine varnishes resulted in dramatic decrease in bacterial counts(29). However, chlorhexidine preparations may induce adverse effect on oral tissues with prolonged use such as burning sensation, taste alteration, teeth and tongue discoloration (30,31). Moreover, microbial resistance has developed due to the excessive use of chlorhexidine in commercial oral care products(32).

The use of natural plant extracts for better oral health generally and caries control specifically have been studied in the literature (33–36) due to their availability, low cost and absence of side effects(37,38). World Health Organization recommended their use due to their proven effectiveness in promoting oral health(39). *Salvadora persica*, commonly known as miswak tree, is the most commonly used natural plant for teeth cleaning in many countries(40). Besides miswak sticks ability to remove plaque mechanically, its ingredient compounds possess an antimicrobial activity with variable organic and inorganic compounds that have been identified in miswak extract, giving it an antibacterial (41,42), antifungal(43), anticariogenic(44), and antiplaque(45) properties. Miswak extract had growth inhibitory effects against the cariogenic bacteria, *Streptococcus mutans and Lactobacillus acidophilus* in vitro(46). Patients who used miswak showed better periodontal status than regular toothbrush users assuming effective protection against periodontopathic bacteria(47).

Moreover, Miswak extract is rich in calcium and phosphorus – with low fluoride content- suggesting remineralizing potential for this plant use(40). In a previous in vitro study(7) miswak varnish resulted in a significant reduction in plaque *S.mutans* countsas chlorhexidine varnish did and was considered a natural replacement to chlorhexidine varnish.

The aim of the current study was to investigate the effect of miswak varnish on the incidence and severity of enamel demineralization around orthodontic brackets compared to fluoride varnish, and to investigate the combined effect of both miswak and fluoride varnishes together on enamel demineralization around orthodontic brackets.

Material and methods

Study design: Prospective randomized controlled clinical study.

Sample size estimation, randomization, and grouping:

According to the results obtained from a previous study (48) and with parameters set at; alpha error ≤0.05, power of 0.8 and 1 degree of freedom; the minimum number of specimens was calculated 29 per group which requires 15 patients per group (2 premolars from each patient). Therefore, 48 participants (16 per group) to compensate for any participants' dropouts or damaged specimens were required as study sample. Patients attending the clinic at department of Orthodontics, Faculty of Dentistry, Alexandria University were screened for eligibility to meet the following inclusion criteria:

- Orthodontic patients that their treatment plan include two upper first premolars extractions.

- Age: 14 - 20 years
- No systemic antibiotics or local antibacterial agents for the last 3 months
- Fully erupted permanent dentition with no missing or impacted teeth.
- Female patients
- Normal salivary flow rate (≥1.0 mL/min) and salivary pH (pH 6.0 - 7.0)(49)

Patients with any of the following criteria were excluded from the participation in the study:

- Smokers
- Patients suffering any systemic disease or mental retardation.
- Patients with extensive dental restorations, active carious lesions or active periodontal disease.
- Patients with initial enamel demineralization (WSLs), enamel defects or anomalies

Every eligible participant (or her guardian for participants younger than 18 years) was supplied with full details about the study aim and procedures and an informed consent was signed before being enrolled in the study. The study protocol had been approved from the ethics committee in Faulty of Dentistry, Alexandria University. (IORG0008839, Ethics Committee No. 0542-11/2022)

Eligible participants were equally allocated in one of 3 groups at random (using a computer-generated randomization table via www.randomizer.com). Allocation concealment was performed by allocating the participants into 3 opaque envelopes, and envelopes were given labels after allocation as follow:

- **(FVgroup):** Fluoride varnish (Fluoro-protector, Ivoclar-Vivadent, Schaan, Liechtenstein) was applied to teeth surface around orthodontic brackets.
- **(MV group):** Freeze dried aqueous miswak extract varnish was applied to teeth surface around orthodontic brackets.
- **(CG group):** Both fluoride and miswak varnishes were applied to teeth surface around orthodontic brackets.

Blinding and intraexaminer reliability:

Patients were blinded to the content of the varnish applied. Enamel microhardness test was carried out by one investigator who was blinded for the teeth belonging to any group. Enamel microhardness test was repeated for all the indentation points in 12 specimens from each group after 2 weeks from the first readings for intraexaminer reliability.

Miswak varnish preparation(50)***:***

Freshly cut Miswak sticks were sundried and ground. Ten grams of ground sticks were soaked at 4° C in sterile distilled water (100 mL). After 48 hours, the mixture was centrifuged and filtered to produce aqueous Miswak extract. Freeze drying of the resultant extract was done to produce freeze dried aqueous Miswak extract powder. The final "Freeze dried aqueous Miswak extract varnish" was produced by mixing 10 grams freeze dried aqueous Miswak extract powder with75 mL of 95% ethanol (solvent), 25ml distilled deionized water, and 20 grams of colophony resin.

Study procedures:

Each eligible patient received periodontal dental care involving scaling and prophylaxis 3 weeks before the bonding appointment. All the patients were provided the same commercially available toothpaste (Signal cavity fighter regular toothpaste) and chlorhexidine-free, alcohol-free mouthwash and were instructed to use the same toothpaste and mouthwash provided to them throughout the whole study period. Patients received a demonstration for oral hygiene practices and were instructed to follow a strict protocol of oral hygiene measures.

Metal brackets and tubes (Unitek Gemini brackets, 3M Unitek Orthodontic Products) were bonded to the buccal surface of the teeth using nonfluoridated composite (Unite; 3M Unitek, Monrovia, Calif). Phosphoric acid 37% was used for enamel etching for 20 seconds and then totally washed from tooth surface by a stream of water. A single coat of adhesive,Transbond XT (3M Unitek; Monrovia,California, USA), was applied to the dried enamel surface using a brush applicator and thinned with a gentle stream of dry air for 10 seconds. Brackets were then applied to the enamel surface and pressed firmly to squeeze all excess composite from underneath the brackets. A sharp dental explorer was used to remove any excess composite around the bracket in all directions. Brackets were confirmed meticulously free of any excess composite. Light cure was done for 10 seconds at each aspectof the bracket: cervical, occlusal, mesial, and distal.

Varnishes were applied, according to the assigned groups, immediately after bonding to the enamel surface around bonded brackets and tubes using applicator brush under partial moisture isolation using cotton rolls and dental high suction and were allowed to dry for 1 minute. Patients were instructed not to eat, drink, or rinse for 2 hours and not to brush their teeth for 24 hours.

The upper first premolar teeth were extracted in every patient after 30 days from bonding appointment by the same oral surgeon. The surgeon was asked to avoid bracket debonding or enamel damage during the extraction procedure. Extracted premolars were stored in refrigerated flasks containing 2% formaldehyde, at neutral pH until the analysis.

The extracted premolars were longitudinally sectioned in a bucco-lingual direction using a double-faced diamond disk under water cooling. Premolars' sectioning was performed by a professional lab technician and were passed throught he centre of the orthodontic bracket and was done carefully to avoid bracket debonding during the sectioning procedure.

Each half-crown section was embedded in acrylic resin and polished with progressively fine abrasive paper discs (320, 600, and 1200 grit) (Fig.1). Enamel demineralization around the brackets was evaluated by cross-sectional microhardness testing machine (HMV-2000, Shimadzu, Kyoto, Japan) using Knoop diamond under 50-g load for 5 seconds.

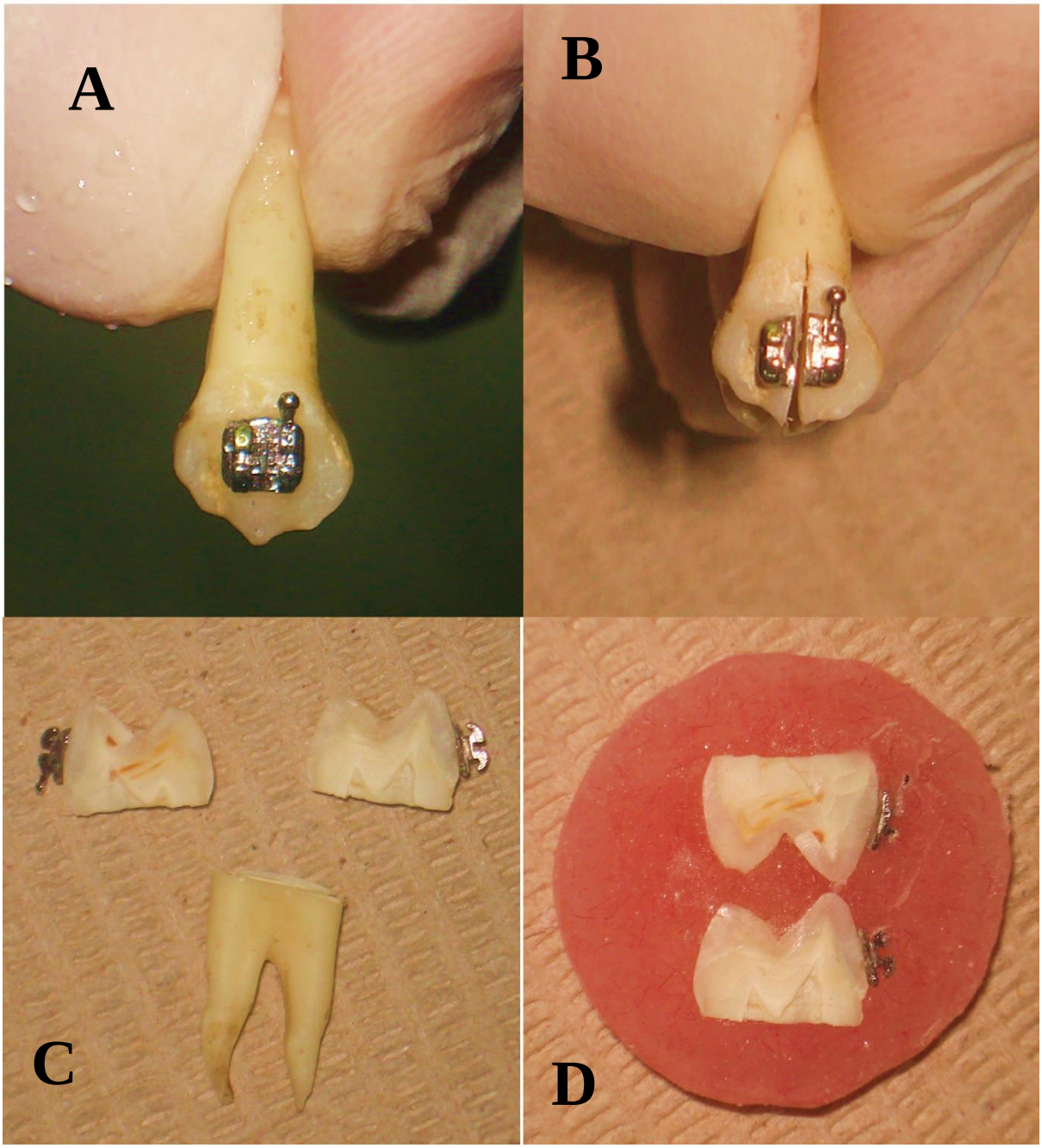

Fig. 1. Specimen preparation for microhardness testing. (A) Teeth extracted and cleaned from blood and soft tissue debris. (B) Teeth and brackets sectioned into two halves in buccolingual direction. (C) Roots removed. (D) The tooth halves are embedded in acrylic resin and ready for microhardness testing.

Enamel microhardness was evaluated in each half crown in 8 different positions. Seven positions were assigned on the buccal surface as follow:

- One point under the bracket (as a control)
- One point at each edge of the bracket (0µm), occlusal and cervical (total 2 points)
- One point at 100µm distance from the bracket edge in both occlusal and cervical direction (total 2 points)
- One point at 200µm distance from the bracket edge in both occlusal and cervical direction (total 2 points)

One position was assigned on the lingual surface: in the middle third of the lingual surface of each half crown (as control).

At each forementioned position, 6 indentations were made at 10, 20, 30, 50, 70, and 90 µm from the enamel surface, giving a total of forty-eight indentations(Fig. 2). The recorded microhardness values found in the 2 half-crowns were averaged and a mean was calculated for every indentation depth.

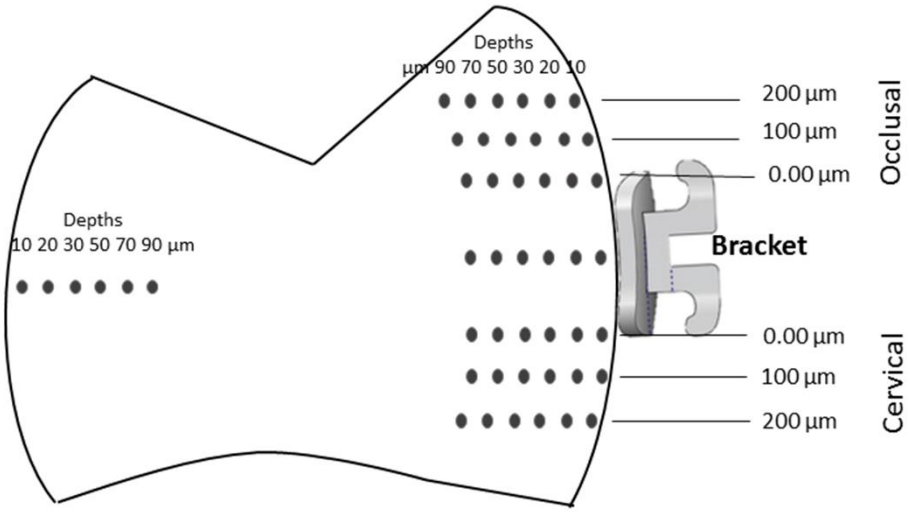

Fig.2 Diagram showing positions and depths of indentations used for enamel microhardness assessment.

Statistical methodology:

Data analysis was performed by using Statistical Package for Social Sciences (SPSS, Version 20.0, SPSS Inc., Chicago, IL, USA). The values of enamel microhardness were presented as (mean ± SD). As the data were normally distributed according to Kolmogorov-Smirnov test. Repeated measures (ANOVA) was used to evaluate the interactions of different enamel treatments (FV, MF and CG), different depths from enamel surface (10, 20, 30, 50, 70 and 90 µm) and different positions from the brackets (on the buccal surface in occlusal and cervical regions at 0, 100µm and 200 µm) and under the brackets and the lingual surface as controls. The Tukey test was used following ANOVA test for multiple comparisons. The statistically significance level was set at $p \leq 0.05$. Intraexaminer

reliability was evaluated by intraclass correlation coefficient (ICC) to assess the reliability of microhardness test.

Results:

Eighty-six patients were examined, and 56 patients met the study inclusion criteria. Eight patients refused to participate in the study. Finally, 48 eligible patients were included in the study, with total 96 premolars (specimens) (fig .3). The age of the patients ranged from 14 years 7 months to 19 years 6 months with mean age 17 years and 1 month. No statistically significant differences regarding the mean age, salivary flow rate, salivary pH and initial enamel microhardness was detected at the start of the trial between different study groups (Table 1). Enamel microhardness in the lingual side at 10µm depth was considered an indication for initial enamel microhardness as enamel of the lingual side of teeth was not bonded or treated with any experimental material. In the fluoride varnish (FV) group, two specimens were excluded, as bracket debonded from one specimen during sectioning and enamel chipped from the surface during sectioning of the other specimen. One specimen was excluded from the combination group (CG) due to enamel chipping from the surface during sectioning. Thereby the total sample consisted of 93 specimens.

The mean intraclass correlation coefficient (ICC) value for the assessment of intra-examiner agreement was 0.87 indicating excellent intra-examiner reliability.

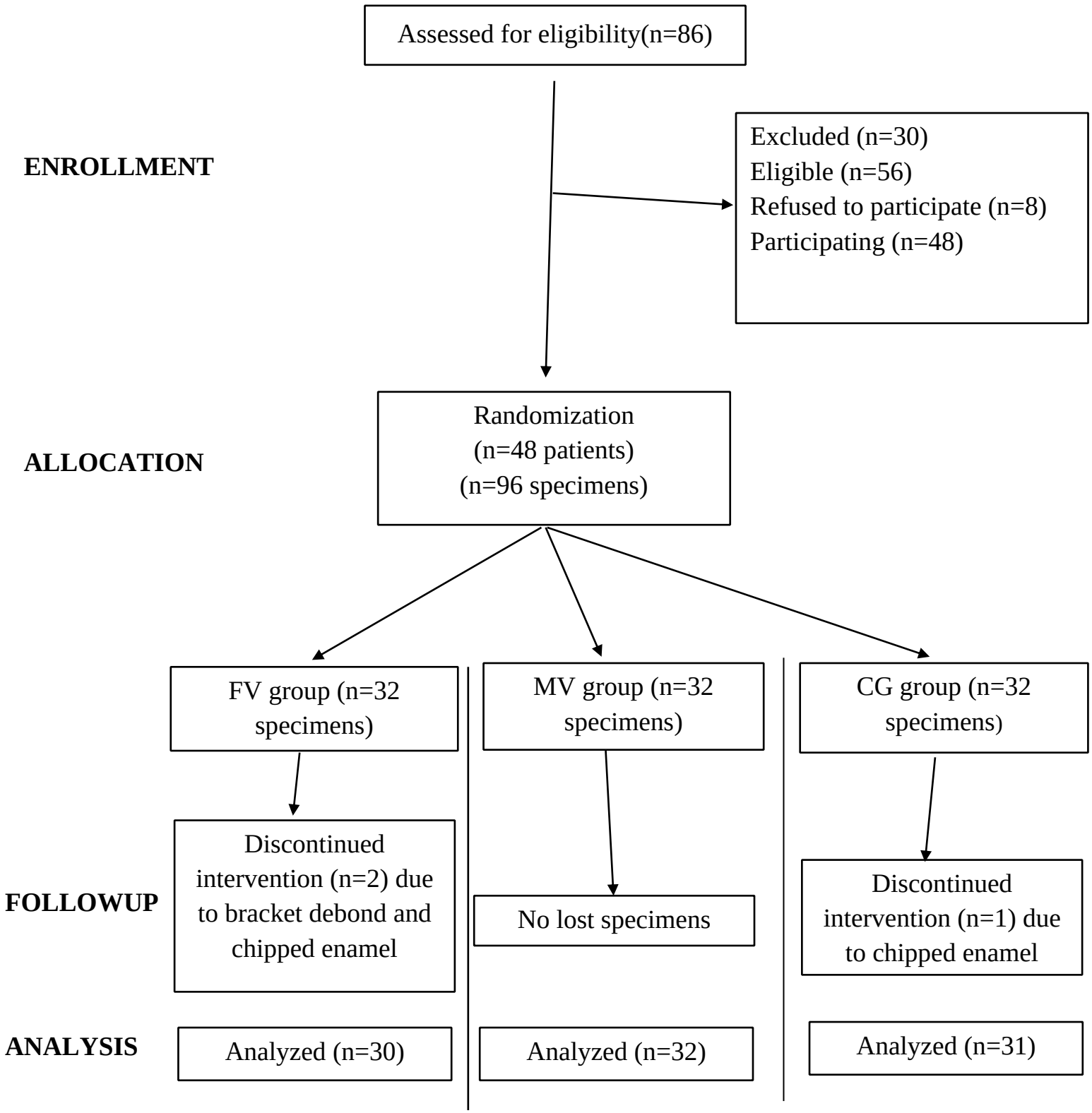

Fig.3 A CONSORT flow chart

Table 1: Descriptive statistics for the sample characteristics in different study groups at start of the trial.

	Group 1 (FV)	Group 2 (MV)	Group 3 (CG)	p-value
Age (Mean ± SD) (n = 48)	17.7±2.1	16.7± 2.1	17.1± 1.6	.806139
Salivary flow rate (n=48)	3.16± 0.64	3.21± 0.76	3.32± 0.75	.84171
Buffer capacity (salivary PH) (n=48)	6.66± 0.34	6.58± 0.47	6.52± 0.57	.718308
Enamel microhardness at lingual at 10µm depth (n=93)	351.8±14.6	351.4±17.3	352.7±18.9	.986279

*Statistically significant at $p \leq 0.05$

The mean of microhardness tested at the distances of 10,20,30,50,70 and 90 µm from the enamel surface in different test group sis shown in table 2. Statistically significant difference between test groups in enamel demineralization was observed at 10µm, 20µm and 30µm from enamel surface. Less demineralization was found in enamel in groups CG and FV in comparison with group MV.

Table 2:Enamel microhardness values (Mean ± SD) for the three test groups at different depths from enamel surface eat the buccal side.

	Group 1 (FV) n = 30	Group 2 (MV) n = 32	Group 3 (CG) n = 31	P value	Multiple comparison (post hoc)		
					FV : MV	FV : CG	MV : CG
10µm	245.45±19.3	219.6±21.4	289.7±23.4	< .00001*	p = .00000*	p = .00000*	p = .00000*
20µm	291.2±23.7	248.4±21.6	332.6±22.5	< .00001*	(p = .00000*	(p = .00000*	(p = .00000*
30µm	325.9±21.4	301.9±23.5	368.4±20.5	< .00001*	(p = .00000*	(p = .00000*	(p = .00000*
50µm	363.7±16.5	361.3±12.9	366.3±14.3	.066138	-	-	-
70µm	379.6±18.3	386.2±15.4	381.4±14.9	.155982	-	-	-
90µm	391±14.6	388.4±12.9	393.1±11.5	.516296	-	-	-

*Statistically significant (P<0 .05)

Statistically significant differences between the three groups were found at distances of 10,20 and 30µm from the enamel surface. The combined group showed the highest microhardness values (least demineralization) followed by the fluoride group. The miswak group had the least

microhardness values indicating the greatest amount of enamel demineralization.

Table 3 shows main effects of the different materials at the different positions. It shows statistically significant difference between the materials in both the cervical region and occlusal region of the bracket up to 100μm distance with greatest mineral loss (lowest microhardness) for the miswak group and least mineral loss (greatest microhardness) observed in the combination group. There was no significant difference between the materials in the hardness observed at both cervical and occlusal regions at distance of 200 μm. Similarly, no significant difference was observed at the lingual surface of the teeth.

Table 3:Descriptive statistics and post hoc comparison for enamel microhardness (Mean ± SD) for the three test groups at different positions: occlusal and cervical to the brackets on buccal side, under the bracket and the lingual surface.

	Group 1 (FV) n = 30	Group 2 (MV) n=32	Group 3 (CG) n=31	P value	Multiple comparison (posthoc		
					FV : MV	FV : CG	MV : CG
Occlusal / 0 μm	329.5±17.7	299.3±19.9	342.5±16.4	< .00001 *	p = .00002*	p = .02261*	p = .00000*
Occlusal /100 μm	322.7±19	305.7±21.5	339.3±20.9	.000032*	p = .022*	p = .02843*	p = .00002*
Occlusal /200 μm	345.5±27.9	341.4±25.4	351.1±22.7	.148594	-	-	-
Under	373.2±19.4	372.5±21.2	374.2±18.9	.935893	-	-	-
Cervical / 0 μm	305.9±20.4	289.7±21.3	328.2±18.1	.000024*	p = .09900	p = .00469*	p = .00002*
Cervical /100 μm	327.1±27.2	307.2±21.8	341.6±20.4	< .00001*	p = .00383*	p = .03324*	p = .00000*
Cervical /200 μm	348.96±19.4	325.42±20.9	363.56±16.3	.00049*	p = .02636*	p = .21742	p = .00035*
Lingual	351.8±14.6	351.4±17.3	352.7±18.9	.986279	-	-	-

*Statistically significant (P <0 .05)

Statistical analysis of the material/position/depth interaction is shown in Table 4. Only three positions (under the brackets, occlusal at 200 μm and lingual) did not show significant difference in microhardness values between the 3 study groups at 10μm depth from the enamel surface. Combined group always had the least amount of demineralization followed by fluoride varnish group and then miswak varnish group with the highest demineralization.

Table 4: Descriptive statistics and multiple comparisons of microhardness for materials and positions at depth of 10 µm.

	Group 1 (FV) n =30	Group 2 (MV) n=32	Group 3 (CG) n=31	P value	Multiple comparison (post hoc)		
					FV : MV	FV : CG	MV : CG
Occlusal 200 µm⁄ 10 µm	260.2±251.1	247.8±15.6	258.9±16.9	.347604	-	-	-
Occlusal 100 µm⁄ 10 µm	239.6±18.1	218.5±13.9	259.2±16.1	.000057*	p = .02467*	p = .04193*	p = .00003*
Occlusal 0 µm⁄ 10 µm	196.5±18.4	175.01±14.1	216.4±19.6	.000126*	p = .03544*	p = .05754	p = .00008*
Under⁄ 10 µm	373.2±19.4	372.5±21.2	374.2±18.9	.935893	-	-	-
Cervical 0 µm⁄ 10 µm	206.1±20.5	154.1±14.9	227.8±17.8	< .00001*	p = .00000*	p = .04444*	p = .00000*
Cervical 100 µm⁄ 10 µm	211.1±21.1	161.9±20	238.1±18.7	< .00001*	p = .00005*	p = .02313*	p = .00000*
Cervical 200 µm⁄ 10 µm	260.2± 12.7	233.4± 14.2	277.6±9.04	< .00001*	p = .00012*	p = .01031*	p = .00000*
Lingual⁄ 10 µm	351.8±14.6	351.4±17.3	352.7±18.9	.986279	-	-	-

*Statistically significant (P <0 .05)

Discussion:

The aim of the current randomized controlled trial was the evaluation of the effect of miswak varnish (MV), fluoride varnish (FV) and the combined use of them (CG) on enamel demineralization adjacent to bonded orthodontic brackets in vivo. The null hypothesis for this study is that the concomitant use of an antimicrobial varnish (miswak varnish) would not have a synergistic effect on fluoride varnish's ability to reduce enamel demineralization around orthodontic brackets.

The current in vivo study has several advantages as (1) the development of the white spots lesions were evaluated in vital teeth exposed to real oral environment other than using an in vitro design that would not result in accurate simulation of all oral environment. (2) The authors used both miswak and fluoride in varnish form to exclude any patient cooperation factors. (3) Microhardness profile,

a reproducible and easy method was used for evaluating enamel demineralization, as a good correlation (0.91) was found between enamel microhardness and mineral content in enamel(51).

However a split mouth design is common in vivo studies evaluating the cariostatic effect of different materials around orthodontic brackets(52,53), it was not used in the current study and each subject in the current study received only 1 tested material according to the group assigned, to avoid any expected diffusion of the test material into saliva that may affect enamel demineralization of other (control) teeth(48). Only females were included in the current study as females showed more compliance and co-operation in following oral hygiene instructions than do males.(54)

Enamel demineralization was detected as early as 1 month following bracket bonding(55,56), therefore, teeth were extracted after 1 month following bracket bonding and enamel demineralization was tested. Two internal controls were used in the current study: under the bracket and at the lingual side. Under the bracket control was used to evaluate the possible effect of acid etching on enamel mineral content(56). However, lingual control was used to evaluate the individual enamel hardness. The microhardness of the enamel under the brackets and on the lingual side in the three test groups was statistically similar (Table 3). This excludes the effect of bonding material or bonding technique used on enamel demineralization and confines the demineralization changes attributed solely to the different varnish material used.

Changes in enamel mineral content was evaluated at 10, 20, 30,50, 70, and 90 µm from the external surface of the enamel. The data in table 2 shows that significant enamel demineralization was observed up to 30 µm depth from enamel surface. This comes in accordance with other previous studies(48,56,57), however, de Moura at al(58) found deeper enamel demineralization extending up to 70µm from external enamel surface. The deeper enamel demineralization lesions found in de Moura study may be attributed to the methodology they followed, where plaque-retention areas inaccessible to routine oral hygiene procedures were created by using oversized bands to allow plaque accumulation between band and the tooth. The results of the current study showed that combined group (CG) always presented the significantly highest microhardness values suggesting less demineralization followed by the fluoride varnish group and then the miswak varnish group came with the least microhardness values.

Table 3 shows reduced enamel microhardness up to 100 µm distance from bracket edge in the occlusal direction and in the cervical direction. In contrary to our findings, Pascotto et al(48) observed reduced enamel hardness in the cervical direction only from bracket edge upto 200µm and Uysal et al(57) found significant reduction in enamel microhardness in cervical direction only upto 100µm only from the bracket edge. This may be explained by patients' inability to efficiently

remove plaque in area close to the orthodontic brackets either cervically or occlusally. As the distance from orthodontic bracket increases, plaque retention becomes less and oral hygiene practices become more effective. Moreover, our study showed statistically significant microhardness differences between the tested materials at the same positions.

Interaction of materials/ positions/ depth of 10µm showed that at 10µm from the surface showed no significant difference between the test groups on the lingual surface, under the brackets and at 200 µm from bracket edge in the occlusal direction (Table 4). These results are in disagreement with Uysal(57) and Pascotto(48), where no significant differences were observed at these studies on the lingual surface only. These studies found a significant difference in enamel microhardness under the brackets, and this could be attributed to the fact that these studies compared different bonding protocols to reduce enamel demineralization. This could be attributed to the different bonding techniques used in these studies, where one study group was bonded using acid etch composite and the other group was bonded using glass ionomer cement with no acid etch. This may results in significant differences in enamel microhardness under the brackets as acid etching can result in average 6% loss of enamel mineral content(56) and subsequently a detectable deference in enamel microhardness between the groups if acid etching was omitted from one group. However, in the current study, all brackets were bonded using acid etch composite resin. Recording no significant difference on the lingual side seems reasonable since these areas were not subjected to demineralization caused by plaque accumulation. The insignificant difference in enamel demineralization observed at 200µm occlusal to bracket edge at 10µm depth in the current study can be explained as occlusal areas are less prone to plaque accumulation and are more accessible to routine oral hygiene measures compared to areas cervical to the bracket with confined space between bracket edge and the tooth gingival margin. Therefore, occlusal enamel especially those areas distant from the bracket edge (200µm) would be less prone to demineralization effects.

The results of the current study suggest that a single fluoride varnish application can significantly reduce the depth of enamel demineralized during the first month of orthodontic appliance wear. These results comes in accordance to Farhadian et al(2) who reported 40% reduction in enamel demineralization lesion depth around orthodontic brackets because of the effect of fluoride varnish. Furthermore, the combined use of miswak as an antimicrobial agent had a synergistic effect on fluoride varnish ability to reduce enamel demineralization as previously concluded by Ullsfoss et al(19) and Øgaard et al(59) when they combined the use of chlorhexidine as an antimicrobial agent with fluoride.

Conclusions:

Within the limitations of this study and the short observation period of 30 days, the combined use of (fluoride and miswak) varnish showed significantly more resistance to enamel demineralization manifested by higher microhardness values compared to fluoride

varnish alone. However, single use of miswak varnish alone resulted in least protection against enamel demineralization in orthodontic patients. This suggests that combined use of miswak and fluoride varnishes in orthodontic patient might provide maximum protection against enamel demineralization.

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